DuraClone IM TCRs Tube, 25 tests, RUO

REF **B53340 – 25 tests**

IFU- B53340-1.0

BECKMAN COULTER

	Specifications of Constituent 1	Specifications of Constituent 2	Specifications of Constituent 3	Specifications of Constituent 4	Specifications of Constituent 5	Specifications of Constituent 6	Specifications of Constituent 7	Specifications of Constituent 8	Specifications of Constituent 9
Specificity	TCRγδ (i.e.TCR gd)	TCRαβ (i.e. TCRab)	HLA-DR	TCR Vδ1 (i.e. TCR Vd1)	CD4	CD8	CD3	TCR Vδ2 (i.e. TCR Vd2)	CD45
Clone	IMMU510	IP26A	Immu-357	R9.12	13B8.2	B9.11	UCHT-1	IMMU 389	J.33
Immunogen	Soluble gamma/delta T-cell receptor	T cell clone	EBV-transformed cell line	Soluble Vγ9 Cγ/Vδ1C	Human thymocytes	Cytotoxic human T clone HLA A2	T cell line + IL2	Soluble γ/ δ T-c receptor	Laz 221 cell line
Isotype	IgG1	IgG1 kappa	IgG1	IgG1	IgG1	IgG1	IgG1 kappa	IgG1	IgG1 kappa
Species	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells						Ascites fluid	Ascites fluid or supernatant of in vitro cultured hybridoma cells	Ascites fluid
Purification	Ion exchange or affinity chromatography	Affinity chromatography	Ion exchange or affinity chromatography	Affinity chromatography	Ion exchange or affinity chromatography	Affinity chromatography	Ion exchange or affinity chromatography	Affinity chromatography	Affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)	R Phycoerythrin (PE)	R Phycoerythrin-Texas Red-X (ECD)	R Phycoerythrin-Cyanine 7 (PC7)	Allophycocyanin(APC)	Alexa Fluor 700 (A700)	Allophycocyanin Alexa Fluor 750 (APC-A750 or AA750)	Pacific Blue (PB)	Krome Orange (KRO)
Excitation peak	488 nm	488 nm	488 nm	488 nm	633 nm	633 nm	633 nm	405 nm	405 nm
Emission peak	525 nm	575 nm	613 nm	770 nm	660 nm	720 nm	783 nm	455 nm	528 nm

For Research Use Only.
Not for use in diagnostic procedures.

BACKGROUND

Mature T cells - as identified by positive surface CD3 staining - express T cell receptors (TCRs) that enable low affinity-recognition of antigens bound to major histocompatibility complex (MHC) molecules.[1] MHC co-binding of either CD4 (MHC class II) or CD8 (MHC class I) enhances and prolongs the engagement with the antigen-presenting cells. Two forms of TCRs are known either consisting of heterodimers of variable α and β protein chains or γ and δ protein chains. Proportions of 95% αβ T cells and 5% γδ T cells are commonly found in blood .The largest subset of γδ T cells can be identified by the IMMU 389 antibody clone recognizing the γδ complex from the Vδ2+ T cell receptor (TCRγδ Vδ2+), being mostly associated with Vγ9.[2] A smaller subset of γδ T cells is staining positive with the R9.12 antibody clone that binds to the Vδ1 segment of γδ T cells (TCRγδ Vδ1+).[3] The relative sizes of these two subsets have been assigned a role in feto-maternal[4] and allograft tolerance.[5]

APPLICATION

The DuraClone IM panels are used to identify cell subpopulations in human whole blood samples by flow cytometry.

The IM TCRs Tube is a 9-color, 9-monoclonal antibody reagent that allows the identification of the TCRs on the surface of mature T cells.
This reagent is intended to be used on a flow cytometer with three lasers:

- A 488 nm laser with detectors dedicated to detection of light scatter (forward and side) and fluorescence emission in the following ranges: 504 – 545 nm, 560 – 600 nm, 605 – 635 nm, 680 – 710 nm and >755 nm.
- A 638 nm laser with detectors dedicated to detection of fluorescence emission in the following ranges: 650 – 670 nm, 715 – 735 nm and >755 nm.
- A 405 nm laser with detectors dedicated to detection of fluorescence emission in the following ranges: 430 – 470 nm and 530 – 570 nm.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by mature T cell lymphocytes. Specific staining of the leukocytes is performed by incubating the sample with IM TCRs Tube. The erythrocytes are then removed by lysis and the leukocytes, which are unaffected by this process of lysis are acquired and analyzed by flow cytometry. The flow cytometer measures light diffusion and the fluorescence of cells; it enables the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light(Side Scatter or SS) and the diffusion of narrow angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage.

KIT BOX CONTENTS

DuraClone IM TCRs Tube, 25 tests, RUO contains the following:

- 25 tests of the DuraClone IM TCRs Tube (i.e. a single tube is a single test), 25 test, RUO
- 3 Compensation Kits, each kit containing 9 tubes, each of a single color; i.e.
  - CD4-FITC
  - CD4-PE
  - HLA DR-ECD
  - VD1-PC7
  - CD4-APC
  - CD8-A700
  - CD3-APC-A750
  - CD4-Pacific Blue
  - CD8-Krome Orange

STATEMENT OF WARNINGS

1. For stability information of DuraClone IM TCRs Tube, refer to the Certificate of Analysis (COA). Discard reagent or compensation tubes, as per applicable regulations.
2. Do not store the reagent tubes or compensation tubes in the refrigerator; do not freeze/thaw the tubes.
3. All blood samples must be considered as potentially infectious and must be handled with care (protective gloves, gowns and goggles must be used while handling blood samples).
4. Discard processed reagent tor compensation tubes and applicable regulations, after sample acquisition and analysis.
5. Minimize the exposure of the tubes to light, especially during incubation of sample stained with fluorescent antibodies or during lysis and after processing of sample, before use.
6. Only calibrated instruments, as per the manufacturer's instructions, should be used.
7. Adhere to the instructions for use while using this reagent.
8. Ensure that the compensation tubes are run in the following order:-
   - CD4-FITC
   - CD4-PE
   - HLA DR-ECD
   - VD1-PC7
   - CD4-APC
   - CD8-A700
   - CD3-APC-A750
   - CD4-Pacific Blue
   - CD8-Krome Orange
9. Seal the zip lock of the pouch containing DuraClone IM TCRs Tube after removing the desired number of tests.
10. Reagent tubes and compensation tubes are to be stored within the sealed pouch containing desiccant packs to prevent the tubes from being exposed to moisture.

STORAGE CONDITIONS

Store the reagent tubes and compensation kit tubes between 20 and 30°C, in a dry place and protect it from the direct exposure to light and moisture.

EVIDENCE OF DETERIORATION

Any damage to the panel or compensation tube may indicate product deterioration and the product should not be used.
Please contact your local distributor or you can contact Beckman Coulter at the following email address:
duraclone-support@beckman.com

INSTRUMENT REQUIREMENTS

This reagent is designed to be used on a flow cytometer such as *Navios, capable of detecting forward and side scatter, and compatible with the emission spectra of the fluorochromes used in the reagent.

SPECIMEN COLLECTION

The whole blood sample should be collected in a blood collection tube containing K_2EDTA.Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. The sample must be stored between 20°C and 30°C.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Blood collection tube containing K_2EDTA
- Calibrated pipettes
- Vortex mixer
- Sheath fluid
- Flow-Check Pro Fluorospheres (REF. A69183) (For Navios alignment verification)
- Flow-Set Pro Fluorospheres (REF. A69184) (For Navios standardization)
- VersaLyse Solution (REF. A09777)
- IOTest 3 Fixative Solution (REF. A07800)
- VersaComp Antibody Capture Beads Kit [Positive Bead] (REF B22804)
- Flow cytometer

PROCEDURE

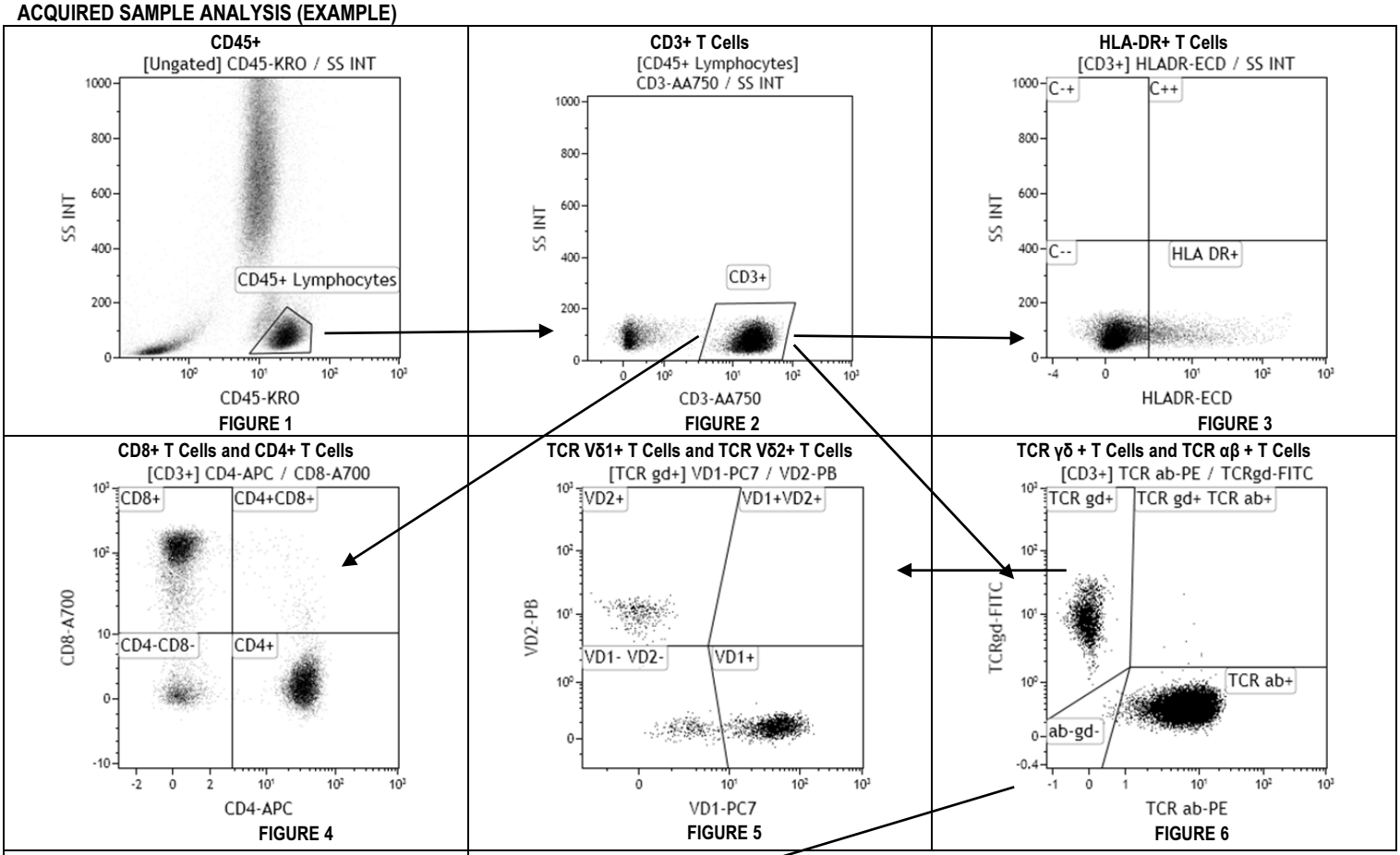
SAMPLE PREPARATION

1. Add 100 µL of fresh whole blood to the dried reagent tube, vortex at high speed for 6-8 seconds and incubate the tube for 15 minutes, protected from the direct exposure to light between 20 and 30°C.
2. Add 2 mL of VersaLyse, vortex the tube at high speed for 1-3 seconds and incubate the tube for 15 minutes protected from the direct exposure to light, between 20 and 30°C.
3. Centrifuge the tube at 200 x g for 5 minutes; aspirate the supernatant, gently tap the cell pellet.
4. Perform a wash step by re-suspending the cell pellet in 3mL 1X PBS and centrifuging the tube at 200 x g for 5 minutes; aspirate the supernatant and re-suspend the cell pellet in 500 µL of 1X PBS containing 0.8% IOTest 3 Fixative Solution. The sample is now ready for acquisition.

COMPENSATION SETUP

1. Stain all the nine single color tubes from the Compensation Kit provided in the DuraClone IM TCRs Tube, 25 tests, RUO with whole blood by following steps 1-4 in the sample preparation procedure. For the VD1-PC7 compensation tube, add two drops of the positive VersaComp antibody capture beads along with 100 µL of blood.
2. For sample acquisition on Navios: The AutoSetup Scheduler on the Navios groups the selected applications for efficient set up in sampling from common compensation samples when scheduling multiple applications and provides the carousel load report to facilitate setting up and loading samples for daily QC. For setting up compensation using AutoSetup Scheduler, refer to the Application Note "Compensation Setup for High Content DuraClone reagents", downloadable from the Beckman Coulter website: www.duraclone.com/im/.
3. For all other flow cytometers, follow standard procedures and instrument manufacturer instructions for compensation setup.

ACQUIRED SAMPLE ANALYSIS (EXAMPLE)



1. Create an appropriate analysis protocol to define the population gates and a series of dual parameter plots for analysis.
2. Set the discriminator on the FS parameter to a value low enough to assure lymphocytes are not excluded from acquisition.
3. Create a CD45-KRO vs. SSC dot plot and apply the leukocyte gate. Create a region to encompass the CD45+ lymphocytes (Figure 1).
4. Create a CD3-AA750 vs. SSC dot plot and draw a region to gate the CD3+ T cells (Figure 2).
5. Create the following dot plots and apply the CD3+ gate on these plots:
   a. Create a HLA-DR-ECD vs. SSC plot and gate the HLADR+ population (Figure 3).
   b. Create a CD4-APC vs. C8-A700 dot plot and draw two regions to gate the CD4+ T cells and CD8+ T cells, respectively (Figure 4).
   c. Create a TCR γδ –FITC vs. TCR αβ –PE plot and gate the TCR γδ + T cells and TCR αβ + T cells (Figure 6).
6. Create a VD1-PC7 (i.e. TCR Vδ1) vs. VD2-PB (i.e. TCR Vδ2) plot, apply the TCR γδ + T cells gate on this plot and delineate the TCR Vδ1 + T cells and TCR Vδ2+ T cells (Figure 5).
7. Create a CD4-APC vs. CD8-A700 plot, apply the TCR αβ + T cells gate onto this plot, and delineate the TCR αβ + CD8+ T cells and TCR αβ + CD4+ T cells (Figure 7).

REFERENCES

1. Structure and function of the T cell antigen receptor. Weiss A. J Clin Invest. 1990 Oct; 86(4):1015-22.
2. Biology of the human γδ T-cell receptor. Porcelli S, Brenner MB, Band H. Immunological Reviews 1991; 120: 137-183
3. Structure of a human gamma delta T-cell antigen receptor. Allison TJ, Winter CC, Fournié JJ, Bonneville M, Garboczi DN. Nature. 2001 Jun 14; 411(6839):820-4.
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5. Characteristics of Vδ1 (+) and Vδ2 (+) γδ T cell subsets in acute liver allograft rejection. Yu X, Liu Z, Wang Y, Wang H, Zhang M, Sun Y, Su H, Jin L, Wang F, Shi M. Transpl Immunol. 2013 Dec; 29(1-4):118-22.

PRODUCT AVAILABILITY

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TRADEMARKS

For additional information, or if a damaged product is received, email Beckman Coulter Customer Service at duraclone-support@beckman.com or contact your local Beckman Coulter Representative.

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